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Organochlorine Compounds in Relation to Breast Cancer, Endometrial Cancer, and Endometriosis: An Assessment of the Biological and Epidemiological Evidence

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ABSTRACT: There is an increasing public and scientific concern that certain chlorinated compounds, recognized as environmental pollutants, may cause estrogen-related neoplastic disease in humans. The main hypothesis has been that certain organochlorines, through their estrogenic actions, might cause breast cancer. From experimental studies, both *in vitro* and *in vivo*, there is evidence that certain organochlorine compounds may cause estrogenic effects, whereas others may cause antiestrogenic effects. In limited studies, some of these compounds in high doses have also been shown to increase and reduce the frequency of estrogen-related tumors in animals. The epidemiological findings regarding the association between organochlorines and breast cancer are inconclusive. However, the largest and best designed study has been interpreted as negative with respect to DDT and polychlorinated biphenyls (PCB) in relation to breast cancer. Associations between organochlorine exposure and endometrial cancer or endometriosis have even more limited empirical basis. The hypothesis that human exposure to environmental levels of organochlorines would favor an estrogenic overactivity leading to an increase in estrogen-dependent formation of mammary or endometrial tumors is not supported by the existing *in vitro*, animal and epidemiological evidence. It can, however, not be conclusively rejected on the basis of available data.

KEY WORDS: chlorinated insecticides, estrogenic effects, PCBs, PCDDs, PCDFs.

I. INTRODUCTION

This review was prompted by an increasing public and scientific concern that certain chlorinated compounds, recognized as environmental pollutants, may cause neoplastic disease in humans. Our objective was to understand whether this hypothesis is well founded biologically and supported by empirical data from humans. We also hoped to reach conclusions that could guide future research. The main hypothesis has been that certain organochlorines, through their estrogenic actions, might contribute to the rising

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influenced by a number of factors including time of day, fore/hind milk (colostrum or early milk has lower fat content than later milk), the number of children previously breast-fed, and duration of lactation.[11] In this review, we will generally report contamination levels in milk fat. However, it is sometimes necessary to refer to levels in whole milk, and all levels reported by the National Human Milk Study are fat-adjusted. Similarly, NHATS has reported adipose tissue levels as lipid-adjusted.[5] With the hope of simplifying reporting, levels in human breast milk, blood, or adipose tissue will be expressed as ppm, ppb, and ppt, rather than as mg/kg, µg/kg, and ng/kg, respectively.

The findings for the individual chemicals are summarized below, but it is useful to point out several general patterns. First, most human exposure results from ingesting contaminated foods, particularly foods with high-fat content (e.g., meats, fish, poultry, dairy products). Second, there was, for most chemicals, a pattern of increasing body burden with advancing age. This is likely the consequence of three factors — accumulated body stores over time, higher levels in older individuals probably because they lived through periods of higher exposure and slower metabolism of some of the compounds by older people. Finally, the geographical variations in body burden vary with the chemical, according to local use and environment contaminations. Table 1 shows use and changed use of selected organochlorines with respect to the U.S. situation.

TABLE 1
Uses of Specific Organochlorine Compounds

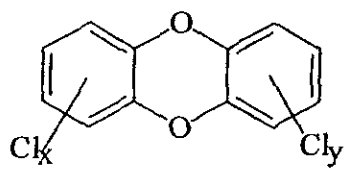
Chemical	Uses	Periods of use in the U.S.
PCDDs PCDFs	Manufacturing and waste byproduct	Inadvertent production, 1930s to present
PCBs	Electric capacitors and transformers	Production began in 1929; peaked in 1970; ceased in 1977
DDT	Agricultural and antimalarial insecticide	Introduced in 1943; production peaked in 1950s; use discontinued in 1972
Chlordane	Insecticide (soil insects and termites)	Introduced in ~~1947~~ (chlordane) and 1952 (heptachlor); production peaked in 1970s; withdrawn in 1980s
Heptachlor	Soil, agricultural, and garden insecticide	
HCB	Agricultural fungicide and manufacturing byproduct	Registered in 1972; use banned in 1975
Aldrin Dieldrin	Agricultural and soil pesticides	Production began in 1950; most uses banned in 1975
BHC	Broad spectrum pesticide	Developed in 1940; use discontinued in 1978
Lindane	Human and livestock scabicide; anthelmintic; agricultural pesticide	Still in use
Mirex	Fire ant insecticide and fire retardant	Used in southeast United States in early 1950s; production ceased in 1967; use banned in mid-1970s
Chlordecone	Agricultural insecticide	Introduced in 1958

2. Polychlorinated Dibenzo-p-Dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs)

The PCDDs and the PCDFs are a series of 210 individual congeners. They are tricyclic aromatic hydrocarbons with a planar configuration (Figure 1). They have never been used commercially but are formed as contaminants in the production of various chlorinated compounds (e.g., 2,4,5-trichlorophenoxyacetic acid and chlorinated phenols) and in different combustion processes.

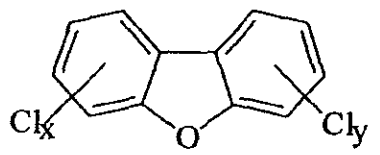
The most well-known and well-studied congener is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Figure 2). TCDD has been shown to act via a cytosolic receptor called the Ah-receptor (AhR).[12] The receptor regulates many cellular proteins, such as cytochrome P450 1A1, and most of the toxic responses to TCDD exposure are believed to be mediated through the AhR.

Although many of the PCDDs/PCDFs are emitted to the environment only congeners with chlorine substitution at least in positions 2, 3, 7, and 8 can be found in analysis of biological material. These stable, lipophilic compounds build up in the food chain, which provides a primary source of human exposure. To handle the risk assessment and management of these compounds, a concept of TCDD equivalencies has been developed. From a variety of generally short-term tests, the toxicity of each individual congener is expressed as a fraction of the toxicity of TCDD. The advantages and limitations of this concept have been discussed extensively.[13,14] Some of the major arguments against the toxic equivalency factor (TEF) concept are that there may not be strict additivity and that toxicokinetic differences between the congeners are not always taken into account. The internationally recommended[15] TEFs for the PCDDs and PCDFs are given in Table 2.



PCDD

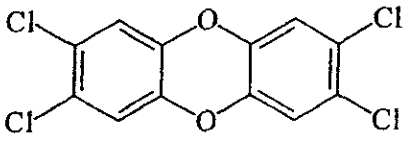
A



PCDF

B

FIGURE 1. The structures of PCDDs and PCDFs.



TCDD

FIGURE 2. The structure of TCDD.

TABLE 2
TEF for Selected PCDDs and PCDFs

Congener	TEF
2,3,7,8-TCDD	1
1,2,3,7,8-PeCDD	0.5
2,3,7,8-substituted HxCDDs	0.1
1,2,3,4,6,7,8-HpCDD	0.01
OCDD	0.001
2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDF	0.05
2,3,4,7,8-PeCDF	0.5
2,3,7,8-substituted HxCDFs	0.1
2,3,7,8-substituted HpCDFs	0.01
OCDF	0.001

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PCDDs and PCDFs are frequently found in human blood samples but typically at very low, previously undetectable levels of 1.0 ppt or less; levels after occupational and accidental exposures (e.g., in Taiwan) may be several hundred times higher.[16] In general, PCDFs are found in substantially lower levels than PCDDs in human samples,[16] although the levels of some 2,3,7,8-substituted PCDF congeners are as high as those of tetra-, penta- and hexa-PCDDs. The less toxic octachlorodibenzo-*p*-dioxin (OCDD) is usually the dominant congener in total PCDD; PCDF patterns differ according to pollution source.[16] Concern has focused on TCDD, which occurs at low levels in humans but is highly toxic.[16] The highest concentrations of TCDD in food typically occur in fish; residues are also high in meat and dairy products.[10]

PCDDs and PCDFs were not assessed in the Total Diet Studies. However, human intake of TCDD in industrialized countries was estimated during the 1980s as 0.1 to 0.2 ng TCDD-equivalents (TEQ; see "TEQ) per day.[10,17,18] Human data regarding TCDD are somewhat limited due to the low body levels of this compound, which increase the expense associated with measurement.[10] NHATS data for 1982 show that TCDD was detected at low levels in the adipose tissue of 76% of the U.S. general population; the highest concentration was 14 ppt.[6] Smaller stud-

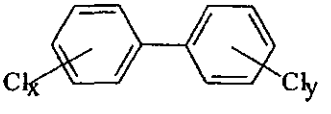
ies have suggested mean levels of about 6 to 12 ppt.[6] A review of numerous small studies of adipose tissue conducted worldwide in the 1980s shows that TCDD was variably detected in 11 to 100% of samples; levels were about 3 to 9 ppt.[10,16] The highest blood levels of TCDD have been found among persons exposed to a chemical cloud arising after an industrial accident in Seveso, Italy.[19] Elevated blood and tissue TCDD levels have also been reported among Vietnam veterans who had direct contact with Agent Orange herbicides.[20] In the U.S., TCDD levels have been shown to increase with age, but racial differences are not apparent.[10]

Worldwide, average levels of total PCDDs in human milk greatly exceeded those of total PCDFs during the 1980s.[16] A recent Swedish study shows that breast milk levels of PCDFs and PCDDs decreased about 50% between 1972 and 1989.[21] TCDD contamination of breast milk has been infrequently assessed, and available data, based on exceedingly small samples, are of dubious value. Nevertheless, reviews of studies published during the 1980s suggest that average breast milk TCDD levels in the industrialized world were about 2 ppt during the 1970s, somewhat lower than in adipose tissue.[10,16] In most studies, TCDD was found in the majority of samples.[16,22] Much higher levels (5- to 20-fold) of TCDD have been found in the breast milk of women inadvertently exposed to TCDD (e.g., Seveso, South Vietnam).[16] The less toxic TCDD occurs at substantially higher levels than the other congeners (150 to 450 ppt).[16]

3. Polychlorinated Biphenyls (PCBs)

The PCBs are a series of 209 congeners of chlorinated aromatic hydrocarbons with the general structure depicted in Figure 3. PCBs were introduced for commercial use during the 1930s and were used as commercial mixtures of the various congeners. They were marketed under trade names such as Aroclor 12xx, Clophen A xx, and Kanechlor xx where "xx" is a code for the percentage of chlorine in the mixture. PCBs had diverse uses in electrical transformers and other electrical appliances. In the U.S., production ceased in 1977[5,10,23] but environmental levels are only slowly decreasing. PCBs have entered the atmosphere, soils, waters, and ultimately the food chain as a result of industrial leakage, waste disposal, and incineration.[19,24] The largest stores are thought to be concentrated in buried landfill dumps, and in the sediments of rivers and the Great Lakes.[25] The entry of PCBs into dairy and beef products has been attributed in part to PCB contamination in protective sealants applied to the interiors of thousands of cattle grain silos in the eastern U.S.[26,27]

The toxicity of individual PCBs is structure dependent. Congeners with no or only one or two chlorine substituents in the *o* positions may assume a planar configuration and thus bind to the AhR and elicit dioxin-like (or TCDD-like) toxicity. With more chlorine substitutes in the *o* position, the rotation of the rings becomes sterically hindered and the dioxin-like properties disappear. The concept of TCDD equivalency has



PCB

FIGURE 3. The structure of the PCBs.

consequently been extended to the dioxin-like PCBs. The internationally recommended TEFs[28] for these PCBs are given in Table 3. All the limitations and uncertainties that are inherent in the concept of TCDD equivalency[13,14,28] also apply to the recommended TEFs for PCBs.

Most of the animal studies with PCB mixtures have been done using commercial mixtures that differ in composition from the PCB mixtures to which humans are exposed through food. Unfortunately, traditional analytical procedures measuring "total PCBs" do not reflect differences in composition. Modern analytical techniques using capillary gas chromatography combined with high-resolution mass spectrometry are able to provide the congener-specific information. However, these methods are expensive and time-consuming.

Human exposure to PCBs is nearly ubiquitous.[5,16] The highest PCB levels have been reported among persons with occupational or accidental exposures, which may result in PCB levels 5- to 1000-fold of those of the general population.[16,23,29] However, most human exposure is due to the ingestion of contaminated fish, dairy products, and meat.[11,30–32] The primary source of dietary exposure varies with the level of food contamination and with dietary practices (particularly fish consumption).[32] Excluding populations with occupational or dietary exposures, PCBs are generally higher in urban, densely populated areas.[16,33] Although PCB production has been halted, PCB products remain in use, with consequent opportunities for continued exposure.[5,16,23]

A review of food studies conducted in the 1970s suggests that concentrations of PCBs in dairy products were high in the U.S. relative to other countries. Worldwide, contamination levels in food have gradually decreased.[32] The U.S. Total Diet Stud-

TABLE 3
WHO/IPCS Recommended Interim TEFs for Human Intake of Dioxin-Like PCBs

	Congener		
Type	IUPAC no.	Structure	TEF
Non-*o*	77	3,3′,4,4′-TeCB	0.0005
	126	3,3′,4,4′,5-PeCB	0.1
	169	3,3′,4,4′,5,5′-HxCB	0.01
Mono-*o*	105	2,3,3′,4,4′-PeCB	0.0001
	114	2,3,4,4′,5-PeCB	0.0005[a]
	118	2,3′,4,4′,5-PeCB	0.0001
	123	2′,3,4,4′,5-PeCB	0.0001
	156	2,3,3′,4,4′,5-HxCB	0.0005
	157	2,3,3′,4,4′,5′-HxCB	0.0005
	167	2,3′,4,4′,5,5′-HxCB	0.00001[a]
	189	2,3,3′,4,4′,5,5′-HpCB	0.0001[a]
Di-*o*	170	2,2′,3,3′,4,4′,5-HpCB	0.0001[a]
	180	2,2′,3,4,4′,5,5′-HpCB	0.00001[a]

Note: The reference value was derived from 2,3,7,8-TCDD (see Table 2).

[a] Based on very limited data.

From Ahlborg, U. G., *Chemosphere*, 28, 1049, 1994. With permission.

ies showed that in adults, estimated PCB intake declined dramatically (but not consistently) from about 7 μg/d in 1971 to 0.1 μg/d in 1987.[32] In 1970 to 1971, nearly half of the food group composed of meat, poultry, and fish was contaminated with PCBs.[4] By the end of the decade, PCBs were found in about 10% of the same food group (mean concentrations were 0.002 ppm).[34] In the 1970s, worldwide intake among nonoccupationally exposed adults in industrial countries (including the U.S.) was estimated at 5 to 15 μg/d.[32]

In the U.S., NHATS (1970 to 1983) data showed that 94% of the general population had been PCB exposed.[5] Adipose tissue levels were typically higher among older (>45) age groups, among men, among nonwhites, and in the northeastern U.S. The 1970s brought a dramatic decline in body burden levels, but not in the frequency of detection. For example, in 1972, 65% of the population had adipose tissue levels in excess of 1 ppm (5% were in excess of 3 ppm); by 1983, only 6% of the population had levels above 1 ppm, but 95% of the population had detectable levels (<1 ppm).[5] Reviews of numerous human studies conducted worldwide suggest that the average PCB burden in human adipose tissue during the 1970s typically ranged from 1 to 5 ppm (fat basis). Blood levels in industrialized countries were about 2 ppb, perhaps somewhat higher in the U.S.

During the 1970s, PCB levels (measured in adipose tissue or blood) clearly decreased in Japan; apparent declines were observed in Canada and Denmark.[33] In the U.S., however, blood levels decreased only gradually over the 1980s.[16,32,33]

During the 1970s and early 1980s, PCBs were present in virtually 100% of breast milk samples in the UNEP/WHO studies.[35] Average PCB levels in breast milk were typically 0.5 to 4 ppm (milk fat) in industrial nations.[11,32,33,35] The high PCB levels found in Sweden and the low frequency of PCB contamination among U.S. women reported by the UNEP/WHO study are not in agreement with numerous previous studies. Unfortunately, such discrepancies are not uncommon, and reflect the variations and distortions inherent in nonstandardized sample collection, and diverse laboratory methods (e.g., differences in quantification and limits of detection).

Lactation is the most important method of eliminating body stores of PCBs, and PCB levels in breast milk are inversely related to the duration of lactation.[11,16] The intake of PCBs of breast-fed children are about 10- to 100-fold greater than of bottle-fed children.[32]

Recently, hydroxylated PCB metabolites have been identified in human serum at concentrations up to 10% of those of 2,2′,4,4′,5,5′-HxCB.[36]

4. TEQ

There are few studies that have measured the levels of individual congeners of PCDDs, PCDFs, and PCBs. WHO/EURO recently finished their second exposure study on levels of PCDDs, PCDFs, and PCBs in human milk (WHO/Euro, in preparation). A total of 19 countries from both within and outside Europe participated and the human milk samples were analyzed with isomer-specific techniques. The results revealed that levels of PCDDs/PCDFs are not increasing in Europe, and that for certain countries a dramatic decrease was evident. The data were also calculated using the TEFs suggested for PCDDs/PCDFs and dioxin-like PCBs.[15,28] The following values (means from 17 individuals) were derived from studies of human milk from the Netherlands: total PCB (measured as sum of PCB 28, 52, 101, 118, 138, 153, and 180) was 253 ppb on a fat basis.

The summarized TEFs (TEQs) were on a fat basis:

PCDD/PCDF	22.4 ppt	(53%)
non-o-substituted PCBs	8.8 ppt	(21%)
Other dioxin-like PCBs	11.6 ppt	(27%)

Hence, about half of the dioxin-like activity from contaminants in human milk might come from the PCB fraction.

In a time trend study in Sweden, Norén[21] analyzed human milk samples from the time period 1972 to 1989 with congener-specific analysis of PCDDs/PCDFs and PCBs. She found that PCBs contributed significantly to the total TEQs in human milk. Her analysis also revealed a decrease in both PCDDs/Fs and PCBs from 1972 to 1985; the levels in 1989, however, were slightly higher than those in 1985.

5. DDT (1,1,1-Trichloro-2-2-bis(4-chlorophenyl)ethane) and its Metabolites

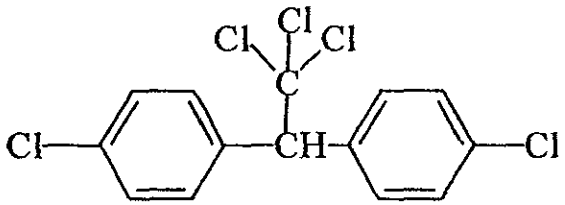
DDT, a chlorinated aromatic hydrocarbon, was introduced as an insecticide in the 1940s but its use in the Western world is now severely restricted. In the U.S., use of DDT reached its peak in the late 1950s. It declined steadily thereafter until its agricultural use was banned in 1972[5] following widespread publicity of adverse effects on wildlife. However, DDT is still used extensively in many developing countries, mainly in programs for the eradication of malaria. The structures of DDT and related compounds and metabolites are given in Figure 4. Technical DDT has the following typical composition[37]:

p,p'-DDT	77.1%
o,p'-DDT	14.9%
p,p'-TDE	0.3%
o,p'-TDE	0.1%
p,p'-DDE	4.0%
o,p'-DDE	0.1%
unidentified	3.5%

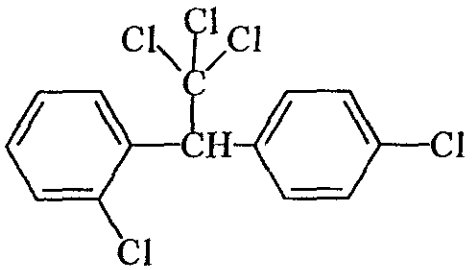
When DDT was given to human volunteers (10 or 20 mg daily oral dosages for 6 months), the levels of *o,p'*-DDT decreased faster than those of *p,p'*-DDT.[38,39] This, in combination with the lower levels of *o,p'*-DDT in the technical product, might explain why very low levels of *o,p'*-DDT have been detected among the general public. Mean breast milk levels of *p,p'*-DDT, *p,p'*-DDE, and *o,p'*-DDT among 418 Canadian women in 1986 were 1.21, 10.33, and 0.31 ppb.[40] The same study also reported a decrease in *o,p'*-DDT from 5 ppb in 1967 to <1 ppb in 1986. These findings are important, as *o,p'*-DDT seems to be the most estrogenically potent individual member of the DDT family (see below "DDT").

p,p'-DDT is metabolized by dehydrochlorination in the environment as well as in humans to the stable lipophilic metabolite *p,p'*-DDE. Therefore, human DDE levels may reflect earlier exposure to DDT and/or direct exposure to environmentally derived DDE, for example, through food ingestion. When analyzing blood levels of DDT/DDE, the ratio DDT/DDE gives an indication of the timing of exposure; higher ratios indicate a recent exposure to the undegraded technical pesticide, whereas lower ratios usually indicate an earlier exposure or chronic exposure through the food chain.

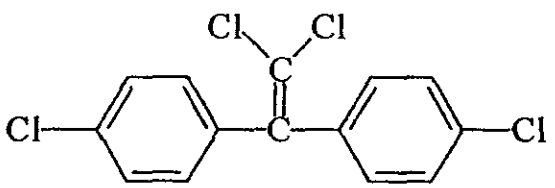
Although there is a global distribution through air, virtually all current human exposure to DDT in Western industrialized countries occurs from ingesting contaminated foods; the highest concentrations of DDT are found in meat, poultry, fish, and dairy products.[4] However, the exposure in some other countries come from spray applications of DDT. Exposure varies with climate; higher levels occur among residents of areas where DDT was used extensively for mosquito control or crop dusting. In general, human levels of DDT/DDE are determined by dietary exposure; however, other factors, including age, gender, disease states, and lactation experience are also influential.[9]



p,p'-DDT



o,p'-DDT



p,p'-DDE

FIGURE 4. The structures of *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE.

Because of their strong lipophilicity, the highest levels of DDT and its derivatives are found in adipose tissue. Fluctuations in body weight (body fat) will influence the concentrations of these chemicals in adipose tissue; specifically, adipose tissue concentrations of DDT decrease with increased body weight and increase with weight loss.[11] A number of studies have shown racial differences in DDT/DDE levels, with markedly higher amounts among blacks, whether based on blood, adipose tissue, or breast milk samples.[5,11,41–45] Racial differences do not appear to be related to proximity to industrial sites; whether they reflect other environmental factors (e.g., diet) or genetic differences in DDT storage or metabolism[46] is unclear. Most surveys suggest that DDT lev-

els are higher among men than among women,[6] which may reflect excretion by way of lactation among women, or other gender differences in storage or metabolism.[5]

The earliest study of food contamination (assessed in restaurant meals) in the U.S. suggested a daily human intake of about 0.2 mg [47]; similar results were reported by numerous small studies of DDT/DDE food contamination conducted in the 1950s and 1960s.[48] A dramatic decline was seen in food contamination levels in the U.S. in the 1970s, subsequent to the ban of DDT in 1972.[48] In 1970 to 1971, among most cities participating in the Total Diet Studies, mean levels of DDT and DDE ranged between 0.004 and 0.009 ppm in meat, fish, and poultry (the most contaminated food groups).[4] By 1979 to 1980, mean DDT levels in this food group had decreased to 0.0008 ppm. However, DDE levels were 0.0048 ppm, only slightly lower than earlier levels.[34] The prevalence of contaminated foods also declined steadily over time. In 1970, DDT was present in about 33% of Total Diet Study foods, but was found in only 4% of foods by 1981 to 1982.[4,34] The same surveys showed DDE present in 37% of foods in 1971, with a less impressive reduction to 22% in 1980 to 1982. The more rapid decline in DDT contamination, relative to DDE, reflects discontinued use of DDT, the environmental conversion of DDT to DDE, and the persistence of DDE.[5] A similar differential reduction in DDT and DDE levels is observed in temporal studies of human contamination levels. Canadian food surveys have typically shown lower levels of DDT food contamination than those observed in the U.S.

Human contamination with total DDT (DDT and its derivatives) peaked during the late 1950s and decreased dramatically in industrial countries subsequent to banning.[6,50] The first report of DDT in human fat was published in the late 1940s.[51] Reviews of data obtained by numerous small studies conducted in the U.S. suggest that total DDT levels (DDT and its derivatives) in human adipose tissue were about 5 ppm in the early 1950s, increased three to fourfold by the end of the decade, then declined over the next 2 decades.[5,6] Based on U.S. general population data provided by NHATS, mean DDT levels in 1970 were 7.88 ppm, but were less than 2 ppm by 1983.[5] This survey (1970 to 1983) also showed that total DDT levels increased steadily from 2.03 to 4.86 to 7.06 ppm across age groups (0 to 14, 15 to 44, >45, respectively, and that average levels in men (5.57 ppm) were slightly higher than those of women (4.73 ppm).[6]

Excellent summaries of international adipose tissue studies are available.[5,9] Relative to the U.S. average adipose tissue levels have been lower in Canada, the U.K., Norway, and Finland. Levels remain elevated in developing countries, for example, in India where DDT remains in current use.[52]

In the U.S., DDT and specific derivatives have also been assessed in blood samples obtained by NHANES II (1976 to 1980).[53] Findings parallel those for body fat. Nearly all samples contained measurable amounts of total DDT.[50] On average, over the survey period, *o,p'*-DDT was found in 33% of samples; *p,p'*-DDE was found in 99%.[6] The frequency of observing *o,p'*-DDT increased nearly fourfold (from 14% to 51%) over the sampled age groups (12 to 24; 25 to 44; 45 to 74); mean levels increased more moderately from about 2.7 ppb to 3.5 ppb with age. In contrast, *p,p'*-DDE was observed in more than 99% of samples regardless of age category. DDE contamination levels were higher relative to DDT, and increased more than threefold (5.9 ppb to 18.3 ppb) across the age groups. The greater frequency and higher levels of DDE, and the more striking increase in DDE across age groups, reflect the degradation of DDT to the more persistent DDE, as well as the heavier exposures and bioaccumulation in older persons.[5,6] Among occupationally exposed cohorts, blood levels of DDT may exceed 50 ppb.[49]

Striking elevations of DDT (>1000 ppb) have also been reported from Triana, AL, where persons living downstream from a defunct DDT manufacturing plant consumed locally caught, highly contaminated fish.[54]

A recent IARC monograph has summarized DDT blood levels in Brazil, Canada, and India.[49] Marked decreases in human levels were observed in Canada and India during the 1970s, and lesser declines were observed in Brazil.[49] However, relative to Canada, levels in India and Brazil are 8- to 15-fold higher.[49] Relatively high levels of DDT have also been reported from Israel.[55]

DDT was the first environmental chemical detected in human milk.[11] The initial report of DDT in human breast milk was published in 1951, about 3 years after the report of adipose tissue contamination; it was based on a small survey of women in Washington, D.C.[56] DDT/DDE was apparently not assessed in the National Human Milk Study; thus, studies of these pesticides in human milk in the U.S. are generally not population based. Reviews of studies conducted in the 1970s show that most mean values of total DDT (fat basis) varied worldwide from 1 to 7 ppm; higher values were observed in Eastern Europe, Spain, East Germany, and in developing nations.[5,11] The validity of these comparisons is subject to the constraints described above.

Levels of DDT and DDE in human milk are similar to or lower than adipose tissue stores.[11] Breast milk excretion of total DDT exceeds current intake, reflecting the depletion of fat stores.[11] The DDT content of human milk is influenced by numerous factors, including mother's exposure, age, weight (and weight loss), parity, and a number of lactation factors, including diurnal fluctuations, fore vs. hind milk, duration of lactation, and lipid content of the breast milk.[11] Although there is mixed evidence concerning an overall urban/rural DDT exposure difference, lactation studies suggest that women living in urban areas have relatively higher levels of DDT (except in rural areas that have received direct contamination through spraying).[11]

6. Methoxychlor

Methoxychlor was introduced commercially as an insecticide in the 1950s. It is metabolized by demethylation and forms phenolic metabolites somewhat resembling diethylstilbestrol (DES). The structure of methoxychlor is given in Figure 5.

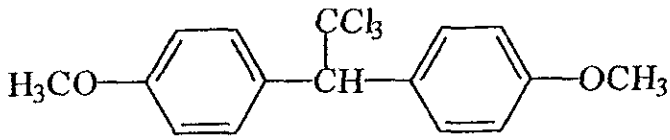
Methoxychlor was identified in about 1% of food composites in the 1970 to 1971 survey of the Total Diet Study, and in about 2% of the composites in the 1979 to 1980 survey.[4,34] The mean concentrations (among positive samples) ranged from 0.002 to 0.006 ppm in the later survey.[34] Based on the 1979 to 1980 survey, estimated adult intake of methoxychlor was 0.007 mg/kg/d.[34] NHATS data suggest low frequencies of detection (2%) that remained fairly stable between 1970 through the early 1980s.[8]

7. Hexachlorobenzene

In the U.S., hexachlorobenzene (HCB) (Figure 6) was registered in 1972 and banned from commercial use in 1975.[5]

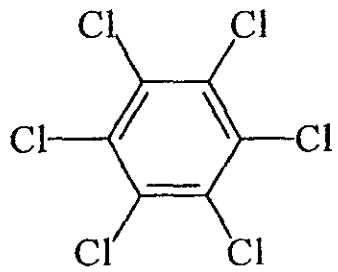
Although HCB had limited use in the U.S., it was used as a fungicide for several decades in many other countries.[5] It is, however, still produced as a byproduct and contaminant in many other chlorinated chemicals including chlorinated solvents. It is slowly metabolized, mainly to less chlorinated benzenes and chlorinated phenols. HCB also occurs in humans as a biological metabolite of hexachlorocyclohexane (HCH), benzene hexachloride (BHC).[5] Most human exposure is probably due to dietary contamination.[5]

HCB was detected in fewer than 3% of food composites in the 1970 to 1971 survey



Methoxychlor

FIGURE 5. The structure of methoxychlor.



Hexachlorobenzene

FIGURE 6. The structure of HCB.

of the Total Diet Study[4]; at the end of the decade, 17% of samples were contaminated.[34] HCB was most frequently detected, and occurred at highest levels, in meat, fish, and poultry.[34] The 1979 to 1980 survey showed that about 75% of these foods were contaminated; the mean concentration was 0.0004 ppm.[34]

In the U.S., NHATS found a median adipose tissue level of 0.037 ppm over the period of 1973 to 1983; HCB was detected in the adipose tissue of virtually all persons.[5] There was neither a consistent decrease nor increase in adipose tissue levels during the survey period; rather, measures fluctuated from 0.02 ppm to 0.05 ppm.[5] Higher levels (about twofold) were consistently noted in the western region of the U.S. Although levels of HCB were typically lowest in the youngest age group (0 to 14), there were no other age differences.[5] Slightly elevated levels were observed among men relative to women, but there were no clear racial differences in HCB levels.[5] Compared with other countries, U.S. levels were relatively low during the 1970s.[5] A review of international data shows that HCB levels in most human adipose tissue studies ranged from about 0.02 to 0.30 ppm during the 1970s.[5]

The NHANES II survey found that about 4% of the population had measurable blood levels of HCB; the mean was 2.1 ppb.[6,50] The frequency and median level of detection varied little over age.[53] There have been few studies of HCB in breast milk in the U.S., but levels in human milk have been evaluated in numerous surveys worldwide.[11] In Europe (1970s), mean levels of HCB typically ranged from 0.10 ppm to 1.0 ppm (milk fat); within this range, the lower levels were observed in Northern Europe.[11] A

substantial increase in milk levels was seen in the U.K. in the 1970s.[16]

8. HCH

HCH or BHC (Figure 7), one of the oldest organochlorine pesticides, was developed in 1940 and used primarily in agriculture and malaria control.[5]

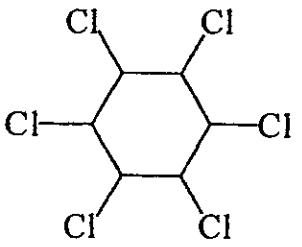
Relative to other countries, HCH was not used extensively in the U.S., and was eliminated from use in 1978; thus, new exposure is unlikely.[5] The most extensive use of HCH occurred in Japan.[9]

Technical-grade HCH contains 65 to 70% α-HCH, 7 to 10% β-HCH, 14 to 15% γ-HCH, and approximately 10% of other isomers and compounds. It has been used as an agricultural pesticide as well as for wood protection. α-HCH and β-HCH are essentially noninsecticidal, but they have occurred as environmental contaminants through the use of technical HCH and are more stable than γ-HCH.

Lindane contains >99% γ-HCH and was introduced as a broad-spectrum insecticide in the early 1950s. Although restricted in some countries, lindane remains in use in the U.S.[5] Unlike most other organochlorines, it is rapidly metabolized and has a comparably short half-life; consequently, it is typically detected in humans only after direct application.[5,16] In addition to agricultural and home gardening use, lindane is marketed for treatment of scabies and (human) lice[9]; contamination occurs through inhalation, dermal exposure, and ingestion.[5]

The early Total Diet Studies measured total HCH; later surveys assessed α-, β-, and γ-HCH. The highest levels of HCH were typically found in dairy products, as well as meats, poultry, and fish; the frequency of detecting HCH appeared to increase over the 1970s.[48] In the first survey, total HCH was detected in 12% of food composites; a decade later, the α-, β-, and/or γ-isomers were identified in a total of 24% of composites.[4,34]

The presence of β-HCH reflects exposure to technical-grade HCH, whereas the γ-isomer indicates lindane exposure.[9] Due to its persistence in humans, β-HCH is of greater concern, and has been more widely evaluated in studies of adipose tissue levels.[5] NHATS data showed that the median concentration level of β-HCH (1970 to 1983) was 0.14 ppm; geometrical mean levels declined over time from 0.37 ppm to 0.10 ppm.[5]



Lindane
(γ-hexachlorocyclohexane)

FIGURE 7. The structure of lindane γ-HCH.

However, there was no temporal change in the frequency of detection, which approached 100% throughout the survey period.[5] Subgroup analyses of NHATS data for the period 1970 to 1983 showed that levels increased across age groups.[5] Slightly higher levels were sometimes (but not consistently) observed among nonwhites relative to whites.[5] In contrast to most organochlorines, levels were slightly higher among women, relative to men, during most but not all survey years.[5] Marked regional differences were apparent. During some survey years, levels in the southern region were twice those of the Northeast, North Central, and Western areas.[5]

After exclusion of countries with unusually high (or low) contamination, total HCH in adipose tissue during the 1960s, 1970s, and early 1980s typically varied between 0.1 and 1.0 ppm.[9] The highest levels of HCH (and β-HCH) were observed in Japan, where adipose tissue contamination levels reached 11 ppm during the mid 1970s, despite a ban on both HCH and lindane in 1971.[5,9]

The NHANES II data showed that about 15% of the population had detectable blood levels of β-HCH and a mean level of 2.4 ppb.[53] Although levels increased only modestly across age groups, differences in the frequency of detection were substantial.[53] Worldwide, total HCH blood levels were highest in Eastern Europe, India, Israel, Argentina, and Japan (20 to 200 ppb).[9] More typical values worldwide range from 1 to 9 ppb.[9]

HCH has been assessed in human milk; a review of studies conducted in the 1970s shows that most samples taken between the late 1960s and early 1980s had mean total HCH levels of 1 to 8 ppb.[9] Higher levels in breast milk have been reported from Latin American countries, Spain, Tunisia, Iraq, and most notably, Japan and India.[9] Levels were generally lowest in Sweden and Denmark, relative to other European countries and to the U.S.

The Total Diet Study reported lindane contamination in 7% of foods in the 1970 to 1971 survey, and this value increased to 11% by 1979 to 1980.[4,34] U.S. population-based data from NHATS suggest that median levels in adipose tissue were about 20 ppb in 1970.[5] Overall, lindane was detected in about 1.8% of the U.S. population; frequency of detection was highest (4%) in the southern U.S. and lowest (0.4%) in the western U.S., where mean levels were also lower (0.01 ppm) relative to other U.S. regions.[5]

NHANES II found that only 0.2% of the population had detectable blood levels of lindane (γ-HCH), the mean level being 2.6 ppb.[6] Lindane has been infrequently evaluated in breast milk. In most industrial countries, levels of β-HCH are higher than those of lindane.[11]

9. Chlordane, Heptachlor, and Heptachlor Epoxide

Chlordane is an insecticide, and the technical product contains a mixture of various chlordane isomers and heptachlor (chlorinated cyclodienes). Chlordane was first produced commercially in the U.S. in 1947; heptachlor (isolated from technical chlordane) was commercially introduced in 1952.[5,57] The use of chlordane and heptachlor in the U.S. has resulted in widespread environmental contamination.[5] These compounds were used primarily to protect buildings, lawns, and gardens from soil insects and termites; heptachlor was also used to control mosquitoes.[5,58] Both compounds have been used to a lesser extent in agricultural settings.[58]

Following EPA regulations of 1983, use of chlordane in the U.S. was restricted to subterranean termite control; all uses were suspended in 1988.[5,58] Heptachlor use has also been subject to restrictions in the U.S. and has been banned in several other countries.[58] The derivatives of these compounds

are very persistent in the environment and in human tissues.[5] Although chlordane and heptachlor were used most extensively in the U.S., both compounds (and to a greater extent their derivatives) have been reported by European surveys of human contamination.[5,11,58] Although exposures in the U.S. have probably occurred as a result of fumigated buildings and from lawn/gardening uses, most exposure is thought to be due to the ingestion of contaminated food.[5]

Heptachlor is metabolized to a persistent metabolite, heptachlor epoxide. Its half-life is considerably longer than that of chlordane. The structures of chlordane, heptachlor, and heptachlor epoxide are given in Figure 8.

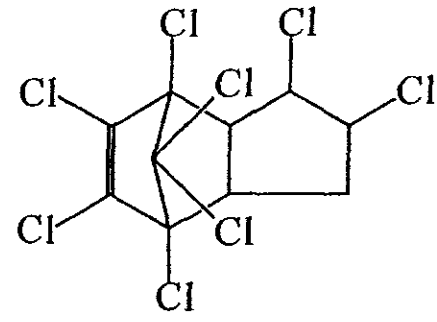
Heptachlor and chlordane themselves have been evaluated infrequently in studies of human exposures. In contrast, human contamination with heptachlor epoxide (a metabolite and environmental oxidation product of heptachlor, and the most persistent metabolite), oxychlordane (an oxidative metabolite of chlordane, sometimes referred to as octachlor epoxide), and *trans*-nonachlor (a component of chlordane and heptachlor) have been more extensively evaluated,[5,58] as summarized below.

Data from the Total Diet Studies showed that heptachlor was detected in less than 1% of foods during the 1970s.[48] Heptachlor epoxide (the most frequently detected metabolite) was found in 10% of foods in 1970 to 1971,[4] and about 12% of food 10 years later.[34,48] In the 1979 to 1980 survey, the greatest frequency of detection (80%) and highest concentrations (0.0009 ppm) of heptachlor epoxide were found in the meat, fish, and poultry composite, followed by dairy products.[34] Oxychlordane and *trans*-nonachlor were found infrequently in food composites in the Total Diet Studies.[5,34]

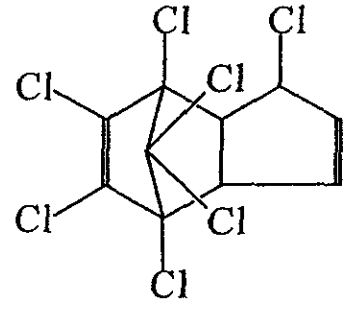
Both heptachlor epoxide and oxychlordane were commonly found in the NHATS (1970 to 1983). The frequency of detection was over 90%.[5] Detection of *trans*-nonachlor was variable. Geometrical mean levels of all three compounds varied modestly during the interval without major trends.[5,45,59] For each of these three compounds, adipose tissue levels were two- to threefold higher among individuals over 45 years old than among those under 15.[6] In general, there seemed to be little variation in levels by race, but men tended to have higher body burdens than women.[45] Sparse international adipose tissue data show widespread low levels of contamination with heptachlor epoxide. On average, levels were about 0.03 ppm in most of the industrialized world during the 1970s.[6]

NHANES II reported detectable heptachlor epoxide in the blood of 4% of the U.S. population, oxychlordane was found in 3.5%, and *trans*-nonachlor in 6.6%.[6,53] Mean levels were 1.8 to 2.4 ppb.[6] Heptachlor epoxide showed increasing levels over age groups, but there were no large differences in levels of oxychlordane and *trans*-nonachlor by age.[53] The heptachlor epoxide levels in NHANES II are higher than those reported by earlier studies, suggesting an increase in heptachlor epoxide during the 1970s.[9]

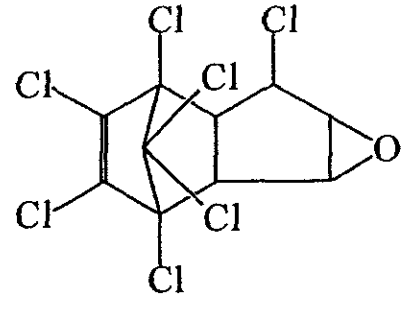
In the U.S. National Human Milk Study, heptachlor was found in less than 2% of human milk samples, but heptachlor epoxide was present in 64% and oxychlordane in 74%. The frequency of detection for these two metabolites was highest in the Southeast (76.7% and 83%, respectively).[60] Mean (fat-adjusted) levels were also markedly higher in the Southeast (128 ppb and 116 ppb, respectively).[60] A review of numerous, usually small, lactation studies conducted in Europe, Africa, and America in the 1970s shows that levels of heptachlor/heptachlor epoxide, measured in milk fat, typically ranged from 0.10 ppm to 0.35 ppm.[11] Studies evaluating chlordane/oxychlordane and *trans*-nonachlor in human milk are few; comparisons are constrained by variations in measurement basis and reporting.[11]



Chlordane



Heptachlor

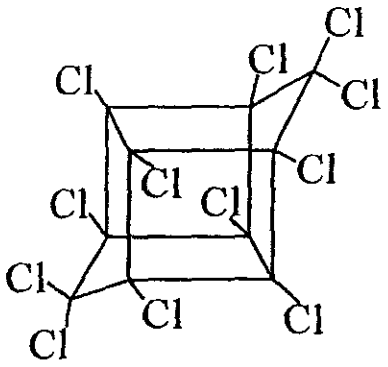


Heptachlorepoxide

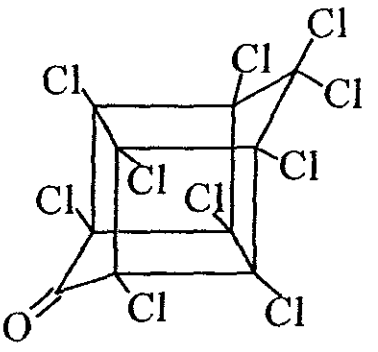
FIGURE 8. The structures of chlordane, heptachlor, and heptachlor epoxide.

10. Mirex and Chlordecone (Kepone)

The structures of mirex and chlordecone are given in Figure 9. Mirex is an insecticide and flame retardant that came into commercial use in 1955. In the U.S., production of mirex ceased in 1967; its use was officially banned in the mid-1970s.[5] Neither mirex nor chlordecone was assessed by the Total Diet Studies.



Mirex



Chlordecone

FIGURE 9. The structures of mirex and chlordecone.

NHATS data (1971 to 1983) have shown a low frequency (<1%) of detectable mirex in the general population of the U.S., which has remained relatively stable over time.[5,6] Interestingly, adipose tissue mirex levels are similar across age categories.[6] A special survey was conducted in states where mirex had been used to control fire ants.[61] On average, about 10% of the population had detectable levels, with a geometrical mean contamination level of 0.286 ppm.[5] The frequency of detection increased from 2 to 15% across age groups; mean concentration also increased with age.[5] A greater frequency of detection (38%) was observed in the East-South Central states, as opposed to the South Atlantic (13%) and west-south central (4%) states.[5] Overall, detection frequency and body burden were higher among nonwhites compared with whites.[5] Relative to women, men were more than twice as likely to have detectable mirex adipose tissue levels, and had somewhat higher mean levels.[5] Mirex was not detected in milk samples assessed by the National Human Milk Study,[60] but has been detected in Canada.[11]

Chlordecone (kepone) was used commercially as an insecticide in the U.S. between 1966 and 1976. Minor amounts of chlordecone may appear as an impurity in mirex. Both compounds are highly stable, lipophilic compounds that are excreted only slowly and mainly unchanged. The biological half-life is several months. Chlordecone has not been evaluated in the U.S. population-based studies.

11. Aldrin and Dieldrin

Aldrin and dieldrin (Figure 10), organochlorine insecticides manufactured since 1950, have been severely restricted or banned in most countries since the early 1970s.

Most aldrin and dieldrin uses were banned in the U.S. in 1975[5,48]; even more extensive restrictions were imposed in 1985, and these compounds are now suspended from use.[5] Both compounds are highly lipophilic. In the environment as well as in the body, aldrin is rapidly degraded to dieldrin. Consequently, aldrin is rarely found in food or in human tissue. The biological half-life of dieldrin is approximately 6 to 12 months. The ratio of dieldrin concentrations in human adipose tissue to that in blood is about 150:1.

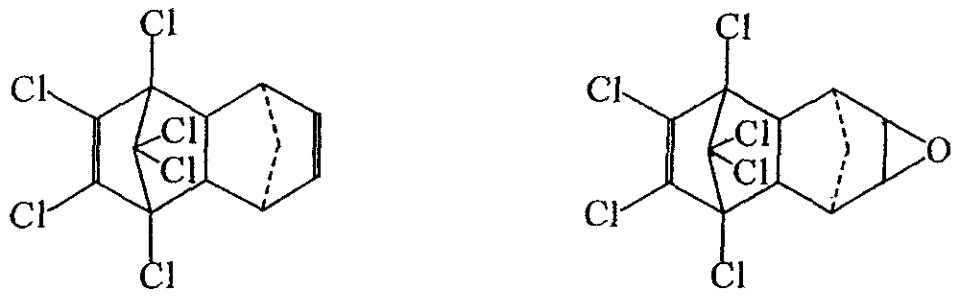


FIGURE 10. The structures of aldrin and dieldrin.

The Total Diet Studies conducted in the U.S. showed a modest decline in the frequency of dieldrin food contamination between 1970 to 1971 (31%) and 1980 to 1981 (21%).[4,34] Mean levels were highest (0.0033 ppm in 1980 to 1981) in the meat, fish, and poultry group, 95% of which was contaminated.[34]

The NHATS showed a steady decline in geometrical mean levels of dieldrin in adipose tissue between 1970 (0.18 ppm) and 1983 (0.06 ppm).[5] Dieldrin levels were higher (0.073, 0.119 and 0.167 ppm) across age groups (0 to 14, 15 to 44, and ≥ 45 years).[6] Kutz et al.[5] summarized studies of international adipose contamination with dieldrin. Overall, average levels in most parts of the world ranged from 0.04 to 0.35 ppm during the 1960s and 1970s. Long-term monitoring of dieldrin in human adipose tissue allows some assessment of temporal trends in Canada and the U.K. In Canada, small surveys showed a decline from 0.17 ppm to 0.04 ppm between 1969 and 1981.[5] Adipose tissue levels in the U.K. decreased from 0.27 ppm to 0.11 ppm between the years 1963 and 1977. Similar declines were observed in Australia after the mid-1960s[6] and in Sweden between 1972 and 1989.[21]

NHANES II (1976 to 1980) showed that 9% of the U.S. population had detectable blood levels of dieldrin; mean levels were 1.9 ppb.[6] The frequency of detection increased from about 1 to 18% across age groups. However, the mean levels did not vary substantially with age.[6] The National Human Milk Study reported that 81% of human milk samples contained detectable dieldrin. The frequency of detection and levels varied from a low of 97.9 ppb among 64% of lactating women in the northeastern U.S. to a high of 242.3 ppb among 89% of samples obtained from the Southeast.[60] In other parts of the world, study results from the 1970s generally showed temporal decreases in frequency of detection and contamination levels of dieldrin in breast milk.[11]

12. Chlorinated Phenols and Phenoxyacetic Acids

Chlorinated phenols and phenoxyacetic acids are much less persistent in the environment than the other chemicals dealt with here. They have not been implicated as interfering in the estrogenic pathways. During

their production, however, some of them are contaminated with PCDDs and PCDFs. Incineration of chlorophenol-treated material may lead to the formation of PCDDs/PCDFs; furthermore, enzymatic synthesis of PCDDs and PCDFs from chlorophenols has been demonstrated.[62] They have thus served as important sources for environmental contamination with PCDDs/PCDFs.

Chlorinated phenols were introduced for use as wood preservatives and pesticides in the late 1930s. Due to the problems with contamination and incineration, their use has been severely restricted or banned in many countries. Chlorinated phenoxyacetic acids were introduced as herbicides during World War II. Only 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has been a substantial problem with respect to contamination. In the 1960s and 1970s, typical commercial formulations could contain up to 0.12 ppm of TCDD.[63] The use of 2,4,5-T has been banned or its use severely restricted in many countries beginning in the 1980s. It should be noted that the use of 2,4,5-T has been much more abundant in the U.S. than in Europe, where use has been predominantly in forestry.

B. Experimental Evidence of Estrogenicity/Antiestrogenicity

1. Introduction

The regulation of estrogenic effects in the organism involves a complex feedback system that may be affected at various levels by exogenous chemicals. Tests to indicate possible estrogenic or antiestrogenic effects may be performed *in vitro* (e.g., measuring binding to the estrogen receptor) or *in vivo* (e.g., measuring increased uterine weight or a variety of other endpoints). Due to toxicokinetic, metabolic, and regulatory factors, a correlation between *in vitro* and *in vivo* findings is not always found.

2. Compounds with Estrogenic Effects

a. DDT

Considerable evidence indicates the estrogenicity of *o,p'*-DDT.[64–66] Weak estrogenic activity in some animal studies has also been noted for *p,p'*-DDT (which comprises 75 to 80% of technical-grade DDT).[32,67]

o,p'-DDT given to immature rats mimicked the effect of estrogen on elevating uterine weight, glycogen content, and activities of various enzymes involved in glycolysis. *o,p'*-DDT also stimulated DNA synthesis and cell division in the uterine luminal epithelium, stroma, and myometrium. The time course of these effects in the stroma and the myometrium was similar following administration of *o,p'*-DDT (250 mg/kg) and 17β-estradiol (50 μg/kg), but despite a 5000-fold higher dose, the maximum response following pesticide treatment was only 70% of that of 17β-estradiol. In the luminal epithelium both compounds yielded the same maximum response, but the time course for *o,p'*-DDT effects was delayed compared with 17β-estradiol.[68] Estrogen receptor retention in uterine nuclei was also prolonged by *o,p'*-DDT. *o,p'*-DDT thus produces the uterine hyperplasia characteristic of estrogens, but the magnitude and timing of the response is dependent on the specific cell type and is different from that of estradiol.[68]

In one study, *p,p'*-DDT, the active pesticide, was also active on uterine enzyme activities, as well as on uterine weight and glycogen content, although to a lesser extent than *o,p'*-DDT.[69] This congener also induced a hypertrophic response in the circular myometrium of immature female Sprague-Dawley rats (a genomic response to estrogen mediated by the cytosol-nuclear receptors) and an edematous response in deep and superficial endometrium (a nongenomic response to estrogen mediated by estrogen receptors located in eosinophils).[70]

However, there have been some studies in which more qualitative differences between *o,p'*-DDT and *p,p'*-DDT emerged. The uterotropic effect of *o,p'*-DDT, but not of *p,p'*-DDT, has been found in minks as well as rats. In ovariectomized Wistar-Furth rats, *o,p'*-DDT (but not *p,p'*-DDT) supported the growth of MT2 mammary adenocarcinoma cells to form tumors in a manner similar to 17β-estradiol.[71]

Both *o,p'*- and *p,p'*-DDT have been associated with effects on hormones and reproductive behavior that imply estrogenic potency of these compounds. *o,p'*-DDT treatment caused precocious vaginal opening, cyclicity, and increases in uterine and ovarian weight in young rats. In ovariectomized rats, it caused cornification of vaginal epithelium as well as induction of typical estrogenic responses in the endometrium. Serum luteinizing hormone (LH), but not follicle-stimulating hormone (FSH), levels were reduced in ovariectomized rats after *o,p'*-DDT treatment. *p,p'*-DDT was also estrogenic in this respect, although less potent.[72] Neonatal exposure to DES decreased basal and gonadotropin-releasing hormone (GnRH)-induced LH secretion in both males and females. *o,p'*-DDT significantly suppressed initial LH levels and blunted GnRH-induced release in males, whereas there was no effect in females. These data show that early exposure to environmental estrogens alters adult rodent pituitary response to GnRH.[73]

Long-term treatment of female rats by *o,p'*-DDT beginning neonatally resulted in permanent sterility associated with advanced puberty, persistent vaginal estrous (PVE) after a period of normal estrous cycles, follicular cysts, corpora lutea reduction, a diminished uterotropic response to administered estradiol, and a slight disturbance in mating behavior. There was an inverse relationship between the age of first vaginal opening (onset of puberty) and dose. It was also found that PVE appeared earlier with higher doses and that the feedback rise in gonadotropin in response to ovariectomy was diminished.[72] In one study, both *o,p'*-DDT and *p,p'*-DDT decreased lordosis behavior at diestrus in adult female F-344 rats, but only *p,p'*-DDT did so at proestrus. *o,p'*-DDT may have altered behavior by disrupting the estrous cycle, whereas *p,p'*-DDT had a major effect on the female's proceptivity and receptivity without modifying vaginal cyclicity. *p,p'*-DDT disrupted sexual behavior at doses of 25 mg/kg, whereas 100 to 200 mg/kg *o,p'*-DDT was required. Because commercially prepared DDT is 75 to 80% *p,p'*-DDT, the authors suggested that some reproductive effects of DDT may have resulted from *p,p'*-DDT rather than from *o,p'*-DDT.[74] However, this is the only study indicating a more pronounced response to *p,p'*-DDT rather than to *o,p'*-DDT.

DDT exposure in mice produced alterations in fertility (increased time to the first litter). *p,p'*-DDT caused a prolonged estrous cycle and a decreased frequency of ova implantation after mating in mice.[72] Discrepancies in results from different studies on reproduction may reflect differences in dose, developmental stage at exposure, exposure duration, and/or degree of *o,p'*-DDT contamination in technical DDT.

There is also evidence of estrogenic effects at the cellular level. *o,p'*-DDT and estradiol increased progesterone binding in the uterine cytosol of ovariectomized rats.[75] The induction of uterine cytosolic progesterone receptor by *o,p'*-DDT, but not by *p,p'*-DDT, was similar to that of estradiol-treated rats.[72] *o,p'*-DDT also markedly elevated rat uterine ornithine decarboxylase (ODC) as did estradiol. For these effects, different DDT analogs decreased in potency from *o,p'*-DDT to *o,p'*-DDD and *p,p'*-DDT. *p,p'*-DDD and *p,p'*-DDE were inactive.[72] *o,p'*-DDT, *p,p'*-DDT, kepone and mirex, but not DES, induced hepatic estradiol-2-hydroxylation, that is reduction in estrogen potency.[76] However, there was no correlation between the ability to induce this enzyme and estrogenic

(or antiestrogenic) properties of a given compound.

The DDT class of compounds can bind to estrogen receptors. *o,p'*-DDT, but not *p,p'*-DDT, inhibited estradiol binding to rat testicular 8S estrogen binding protein in a competitive manner. *o,p'*-DDT also inhibited binding of 5α-dihydrotestosterone to specific receptor proteins in prostate cytosol from the rat.[72] *o,p'*-DDT and *o,p'*-DDE, but not *o,p'*-DDD, *m,p'*-DDD, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD or DDA, inhibited binding of estradiol to rat uterine cytosolic estrogen receptor *in vitro*.[68,77–80] The binding inhibition was competitive in nature and was not caused by destruction of the receptor.

In immature rats, *o,p'*-DDT can actually "translocate" the estrogen receptor from the cytosolic to the nuclear compartment in a manner similar to that of estradiol. *p,p'*-DDT and *p,p'*-DDE were inactive in this respect.[72] McBlain[81] showed that the levo, but not the dextro, enantiomer of *o,p'*-DDT inhibits the estradiol binding to the estrogen receptor. The inhibition of estrogen binding is correlated with uterotropic activity *in vivo*.[82] There was an increased uterine DNA synthesis in rats treated with *o,p'*-DDT. Evidence that estradiol and *o,p'*-DDT interact with a common receptor was obtained when the maximal effects on uterine weight and DNA synthesis by these compounds were found to be nonadditive.[72] *o,p'*-DDT also interacts with cytosol estrogen receptors in the hypothalamus and pituitary of female rats.[83]

Incubation of *o,p'*-DDT with liver microsomes does not enhance the apparent affinity of *o,p'*-DDT for the estrogen receptor, indicating that metabolism is not required for the DDT analog to interact with the receptor. Of 20 metabolites of *o,p'*-DDT, only 3-OH-*o,p'*-DDT was shown to be more active than *o,p'*-DDT itself. This metabolite appears in the feces of rats and chickens.[67] When different *o,p'*-DDT analogs were tested for effects on rat uterine glycogen, it was demonstrated that a stable ethane chain is necessary.[84] Activity of *o,p'*-DDT was retained if a hydroxy or methoxy group was substituted in the 3 or 4 position.

Not all estrogenic effects of DDT congeners are mediated by the intranuclear estrogen receptor, among them initiation of implantation. *o,p'*-DDT initiated implantation and maintained pregnancy for at least 3 d in the progesterone-primed hypophysectomized adult Holtzman rat. When administered at the time of mating or early after fertilization, the same dosage (100 to 200 mg/kg) caused a loss of preimplantation embryos. The results are consistent with the interpretation that *o,p'*-DDT has multiple estrogenic actions that may involve more than one kind of receptor.[85] When progesterone-primed, delayed-implanting hypophysectomized rats were exposed at day 8 of pregnancy, *o,p'*-DDE was about as potent in permitting initiation of implantation as *o,p'*-DDT. *o,p'*-DDD and *p,p'*-DDT were nearly devoid of estrogen activity.[86]

b. Methoxychlor

Methoxychlor (*bis-p*-methoxy DDT) is uterotropic in immature rats, but it is less potent than *o,p'*-DDT or technical-grade DDT. Methoxychlor also inhibits the uptake of estradiol by rat uteri and inhibits estradiol binding to rat uterine cytosolic estrogen receptor.[72]

The technical-grade methoxychlor was more potent than a purified preparation.[87] The purified methoxychlor did not suppress estradiol binding to the estrogen receptor and did not "translocate" estrogen receptor into the nuclear compartment of rat uteri *in vitro*. However, when liver microsomes fortified with NADPH were added to a similar preparation, polar metabolites of methoxychlor were formed and translocation of estrogen receptor occurred. With respect to elevation of uterine ODC activity and wet weight, the purified preparation was only slightly less estrogenic than the unpurified

methoxychlor. Methoxychlor is demethylated to mono- and *bis*-phenol derivatives *in vitro* and *in vivo* and these metabolites are structurally related to DES.[67] The *bis*-phenol metabolite (HPTE) was about 100-fold more active than purified laboratory-grade methoxychlor. For rat uterine estrogen receptor binding *in vitro*, HPTE was about 100 times less active than DES, about 10 times more active than *o,p'*-DDT and slightly more active than the monophenolic derivative of methoxychlor.[72,88] Methoxychlor was about as potent as *o,p'*-DDT in initiating implantation in pregnant rats.[86]

c. Kepone/Mirex

Chlordecone (kepone) competes with estradiol for binding to the estradiol receptor *in vitro* and *in vivo*.[89] It has an estrogen-like action in the uterus of the rat and mouse and in the quail and chicken oviduct. Kepone is even more potent than *o,p'*-DDT in estrogen receptor binding *in vitro*, causing uterotropic effects *in vivo* and initiating implantation in pregnant rats.[86,90] Kepone has an estrogen-like effect on quail testes and abnormal spermatogenesis has been observed in victims of kepone toxicity at a production facility in Virginia.[72] In a study on male rats, chlordecone did not mimic estrogen in its effect on the reproductive system at the dose levels tested.[91] The kepone analog mirex was not uterotropic in the rat.[72]

d. HCB

HCB caused a dose-dependent suppression of serum progesterone concentrations during the luteal phase of the menstrual cycle in cynomolgus monkeys.[92] However, in female rats, serum concentrations of progesterone were elevated after HCB treatment and no estrogenic effects were found.[93]

e. PCBs

Some PCB congeners appear to have estrogenic properties. Due to structural similarities to estradiol, hydroxylated PCBs that are also conformationally restricted due to *o* substitution might be effective in binding the estrogen receptor.[94] In addition, Jansen et al.[95] showed that certain PCB congeners, putative PCB metabolites, and complex PCB mixtures (<48% chlorine content) were estrogenic and bound competitively to estrogen receptors leading to increases in uterine weight in rats. The uterotropic activity of different commercial mixtures of PCBs (Aroclors) as well as individual PCB congeners has also been studied in the immature rat.[96] All of the Aroclor mixtures (1221, 1232, 1242, 1248, 1254, and 1260) except Aroclor 1016 produced at least one of the effects measured in the uterus (increased weight, water content, or glycogen content). Of the PCB congeners studied (mono, di, tetra, and hexa), 2-MCB and 2,2'-DCB were active, but the results of the other congeners were inconclusive.

The estrogen receptor binding activity of ten different hydroxylated PCBs has been measured using estrogen receptors from the uteri of ovariectomized mice.[97] The relative doses required for equal effect were compared to that of estradiol, which was set to 1. DES was more potent than estradiol (factor 0.4). The most potent hydroxylated PCB was 4-OH-2',4',6'-trichlorobiphenyl (TrCB) (42 times less potent than estradiol). Nonchlorinated hydroxylated biphenyls were inactive. The results agree with the *in vivo* uterotropic potency for two of the hydroxylated PCBs in the adult ovariectomized CD-1 mouse; 4-OH-2',4',6'-TrCB, but not 4,4'-OH-3,3',5,5'-tetrachlorobiphenyl (TeCB) was uterotropic.[97] Conformationally restricted hydroxylated PCBs were particularly effective binding ligands for the estrogen receptor. The enhanced binding activity of the *o*-substituted biphenyls is believed to be as-

sociated not only with the close match of the phenolic ring with the A-ring in estradiol, but also with the reduced conformational flexibility and possibly increased hydrophobic bulk of the molecule brought about by the presence of the *o*-chlorine and other substituents.[97]

The importance of these findings is difficult to evaluate at present but nine hydroxylated PCBs have been identified in plasma of humans environmentally exposed to PCBs.[36] The identified metabolites had five to seven chlorine atoms and 4-OH-2,2′,3,4′,5,5′,6-HpCB was the major metabolite. The estrogenic potency of these metabolites must, however, be further investigated.

There are also estrogen-like effects of PCBs on hormone levels and reproductive events. Ovulation was blocked in rhesus monkeys treated with the PCB mixture Clophen A30 (three of four monkeys) or HCB (one of four monkeys) during one estrous cycle.[98] Low estrogen levels were found during the anovulatory cycles, whereas the levels of LH and FSH did not seem to be changed directly by the treatment. Treatment of adult female rhesus monkeys with Arovlor 1254 for 2 years did not affect serum estrogen or progesterone levels during two different estrous cycles.[99] There was no effect on the menstrual cycle length, but menses duration was marginally longer in the high-dose group.

Aroclor 1221 induced uterine growth, precocious puberty, PVE and anovulation in rats treated during neonatal development,[100] but the more highly chlorinated PCB products, Aroclors 1242, 1254, and 1260, did not produce these effects. In the immature female Sprague-Dawley rat, Aroclor 1242 as well as 2,2′,5,5′-TeCB and the putative PCB metabolite, 4-OH-2′,4′,6′-TrCB were estrogenic in increasing the uterine weight.

The offspring of Sprague-Dawley rats dosed with DES (10 μg/kg/d) or 3,3′,4,4′-TeCB (3 mg/kg/d) at days 6 to 18 of pregnancy were studied.[101] Both compounds caused resorptions, but only TeCB caused malformations. Monoamine oxidase development in the brain was not altered by DES, but TeCB depressed the activity both in the brain and in the liver. Both compounds depressed male serum testosterone levels in 35-d-old rats and this effect remained after puberty (d 70) in TeCB-treated rats but not in DES-treated rats. Female testosterone and male and female serum estrogen levels were not affected by either compound. The disparate response to DES and TeCB on the enzymes and hormones studied suggests that the toxic effects of these compounds are mediated by quite different biochemical mechanisms.

Guinea pigs treated with the commercial PCB mixture Clophen A50 during gestation had increased plasma concentrations of 15-keto-13,14-dihydroprostaglandin F-2α, estrone sulfate and 17β-estradiol during the later stages of gestation.[102] No changes were observed in plasma progesterone concentrations.

The commercial PCB mixture Aroclor 1242 affected basal LH and FSH secretion by pituitary cells *in vitro*. It also altered pituitary responsiveness to GnRH. Some of these effects were similar to those of estradiol; however, they occurred only at doses 4 to 5 orders of magnitude higher than estradiol. Other effects were clearly different than those observed with estradiol.[95]

In addition to the estrogenic effects of PCBs, several PCB congeners bear structural similarities to TCDD, which exhibits antiestrogenic activity *in vitro* and *in vivo*. The antiestrogenic effects of PCBs are described later.

f. Conclusion

o,p′-DDT has been shown to mimic the action of 17β-estradiol in various species. Some (perhaps all) of the effects are caused

by the binding of *o,p'*-DDT to the estrogen receptor. Other organochlorine compounds have also been shown to cause one or more estrogenic effects, namely *o,p'*-DDE, *o,p'*-DDD, *p,p'*-DDT, 3-OH-*o,p'*-DDT, methoxychlor (its demethylated metabolites), kepone (chlordecone), and PCB (some congeners and/or their hydroxylated metabolites). These compounds are much less potent (at least 2 orders of magnitude, e.g., *o,p'*-DDT, chlordecone) in this respect than 17β-estradiol. However, compounds such as chlorinated hydrocarbons with long biological half-lives persist and accumulate in the food chain and might reach high concentrations and hence elicit a significant estrogenic effect.

It is presently not possible to assess whether the levels of these organochlorines in the environment (human food) are high enough to cause estrogenic effects in humans. This problem is further complicated by the presence of similar organochlorines (e.g., dioxins, dioxin-like PCBs) with antiestrogenic properties. Moreover, there is no available information regarding the relative potency of many of the compounds structurally related to those that have been shown to possess weak estrogenic potency (e.g., HCH, chlordane, heptachlor, heptachlor-epoxide, and dieldrin).

3. Compounds with Antiestrogenic Effects

a. In Vivo Data

There is considerable evidence that TCDD has antiestrogenic properties. Kociba et al.[103] first reported that TCDD inhibited the development of spontaneous mammary tumors in female Sprague-Dawley rats. Similar findings have recently been reported also in mice[104] and dimethylbenzanthracene-treated rats.[105] It was later shown that TCDD and related halogenated aryl hydrocarbons elicit a diverse spectrum of antiestrogenic responses in rodents and in human breast cancer cell lines.[106] TCDD also has a broad spectrum of antiestrogenic responses in the female Sprague-Dawley rat uterus. For example, TCDD inhibited both constitutive an 17β-estradiol-induced uterine wet weight increase as well as peroxidase activity, and diminished nuclear and cytosolic estrogen and progesterone receptor levels; also TCDD inhibited the 17β-estradiol-induced progesterone receptor binding activity, epidermal growth factor (EGF) receptor binding, EGF receptor and EGF receptor mRNA levels, and c-fos protooncogene mRNA levels.[107-109]

TCDD also caused a significant decrease in 17β-estradiol-induced uterine wet weight in weanling C57BL/6 and other strains of mice,[106,110,111] and caused approximately a 30% decrease in the maximum binding capacities of both the hepatic glucocorticoid and estrogen receptors in Ah-responsive mice.[112] The differences between Ah-responsive and nonresponsive mice appear to reflect differences in the affinity of the AhR. Dose-response curves indicated that there was a difference in the responsiveness of the hepatic estrogen receptor to TCDD in the two congenic strains. These results indicate that the AhR regulates the effects of TCDD on the binding of estrogen to the hepatic estrogen receptor.[113]

Estradiol can antagonize the decreased uterine weight in CD-1 mice induced by TCDD and the decreased uterine weight correlated with the decrease in uterine and hepatic estrogen receptors, without a concomitant decrease in circulating estrogen levels.[114] Doses of TCDD that caused malformations/embryotoxicity in fetuses of Holtzman rats did not affect serum estradiol levels during pregnancy.[115] The antiestrogenic activities were dependent on age. In Sprague-Dawley rats, effects on uterine weight and progesterone receptor levels were seen in 4-week-old and 10-week-old rats, but not in 3-week-old rats. There was a cor-

relation between the age dependent antiestrogenic potency of TCDD and the levels of the uterine AhR.[111]

Vaginal estrous cyclicity and behavioral estrous cyclicity (running wheel activity) were not affected by perinatal exposure to TCDD from puberty to day 150 in female LE rats.[116] TCDD inhibited implantation initiated by estrone in progesterone-primed hypophysectomized rats.[86] TCDD treatment of postpubescent male rats increased the potencies of both androgens and estrogens as feedback inhibitors of LH secretion.[117,118] These increases were due, at least in part, to a TCDD-induced alteration in the regulation of pituitary responsiveness to GnRH.

The effect of TCDD on estrogen receptors has been investigated in several studies. TCDD treatment did not alter the affinity of hepatic and uterine estrogen receptors in guinea pigs, rats, or hamsters exposed to about equally toxic doses. The density of hepatic estrogen receptors was decreased in the guinea pig and in the rat, but not in the hamster. In control animals, the density of uterine estrogen receptors correlated inversely with the LD_{50} for TCDD in these three species.[119]

For different PCDDs, there was an excellent correlation between the binding affinities of these congeners for the AhR and their ability to down-regulate uterine and hepatic estrogen receptor levels.[120,121] 6-Methyl-1,3,8-trichlorodibenzofuran (MCDF), like TCDD, acts as an antiestrogen in the female rat, causing a dose-dependent decrease in uterine and hepatic cytosolic and nuclear estrogen and progesterone receptor levels.[122,123]

Both MCDF and TCDD, as well as 6-*t*-butyl-1,3,8-trichlorodibenzofuran, but not 6-cyclohexyl-1,3,8-trichlorodibenzofuran, antagonized the estradiol-induced increase in uterine wet weight.[124] As the potency ratios for antiestrogenicity and enzyme induction between MCDF and TCDD differ considerably (300 to 700 and >150,000, respectively), the antiestrogenicity of TCDD and related compounds may not be due to increased metabolism of estradiol. Cotreatment of female rats with TCDD and MCDF showed that MCDF partially antagonized the enzyme induction by TCDD.[122] In contrast, 6-nitro-1,3,8-trichlorodibenzofuran (NCDF), a weak AhR agonist and inducer of ethoxy resorufin *O*-deethylase (EROD), caused a dose and time-dependent increase in uterine wet weight and cytosolic and nuclear estrogen receptor and progesterone receptor levels in immature female Sprague-Dawley rats.[125] NCDF did not increase rat uterine peroxidase activity or EGF receptor binding activity. TCDD inhibited the uterotropic effects caused by NCDF but did not affect the NCDF-induced uterine estrogen and progesterone receptor levels.

Exposure to other organochlorines may also lead to antiestrogenic effects. For example, chronic exposure of female Fischer 344 rats to lindane caused a delay in vaginal opening, disrupted estrous cycling, and reduced pituitary and uterine weights and it was suggested that lindane may be antiestrogenic rather than estrogenic.[126] The dioxin-like PCB congener, 3,3′,4,4′-TeCB antagonized the uterotropic effect of estradiol in the immature female Sprague-Dawley rat.[95] This antagonizing effect was not seen with the commercial PCB mixture, Aroclor 1242. 3,3′,4,4′-TeCB was not uterotropic as Aroclor 1242, the *o*-substituted PCB congener, 2,2′,5,5′-TeCB, or the putative metabolite 4-OH-2′,4′,6′-TrCB.

b. In Vitro Data

In Ah-responsive MCF-7 human breast cancer cells, TCDD inhibits 17β-estradiol-induced cell proliferation and causes a rapid down-regulation of the nuclear estrogen receptor binding activity and its immunoreactive protein.[127] TCDD also decreased the binding of estrogen to an estrogen-respon-

sive element.[127] Estrogen causes enhanced secretion of the 34-, 52-, and 160-kDa proteins. The two former proteins have been identified as cathepsin D (an aspartyl protease) and procathepsin D, and the presence of these two proteins in primary human breast tumors is correlated with a poor prognosis for disease-free survival. TCDD significantly inhibited the estrogen-induced secretion of all three proteins as well as cell proliferation and DNA content in MCF-7 cells, but not in the Ah-nonresponsive MDA-MB-231 human breast cancer cell line.[106,128,129] TCDD also inhibited the proliferative effects of mitogens other than estrogen (insulin-like growth factor-1 [IGF-1], transforming growth factor-α [TGF-α] and EGF in MCF-7 cells.[130]

With few exceptions, the order of potency for many PCDD, PCDF, and PCB congeners as inhibitors of the formation of intracellular cytosolic 52- and 34-kDa proteins and mitogen-induced cell proliferation in MCF-7 human breast cancer cells parallels their relative activities as agonists for other AhR-mediated responses, such as CYP1A1 enzyme induction, and their competitive binding affinities for the AhR.[131] Also, the nondioxin-like AhR agonist 3-methylcholanthrene antagonized estrogen-induced receptor binding and cell proliferation in MCF-7 cells.[132] TCDD suppresses the 17β-estradiol-stimulated secretion of tissue plasminogen activator activity in MCF-7 cells, but not in the estrogen-independent breast cancer cell line MDA-MB-231.[133] The results of these studies support the role for the AhR in mediating the inhibition of several of the 17β-estradiol-induced effects in MCF-7 cells.[134,135] However, this does not seem to be true for MCDF, which is a much more potent antiestrogen than an inducer of CYP1A1 enzyme activity. Thus, the antiestrogenic effects of MCDF does not require the induction of the CYP1A1 gene expression but may involve the induction of other genes.[136] TCDD completely inhibited the 17β-estradiol-induced metabolism of glucose to lactate in MCF-7 cells and this effect occurred earlier than the induction of monooxygenase activity.[137]

TCDD suppressed postconfluent multicellular foci formation in MCF-7 cells with an EC_{50} value between 10 to 100 p*M*.[138] Cotreatment of MCF-7 cells with TCDD plus ^{3}H-17β-estradiol did not affect the levels of occupied nuclear estrogen receptor complex. However, pretreatment of the cells with TCDD 6 or 12 h prior to addition of the radiolabeled hormone resulted in a significant down-regulation of occupied nuclear estrogen receptor levels.[139] The concentration-dependent decrease in estrogen receptor binding was paralleled by a decrease in immunoreactive estrogen receptor protein. The same effects on estrogen receptor levels were seen in mouse Hepa lclc7 cells, but not in various Ah-nonresponsive mutant cell lines.[136,140]

c. Mechanisms of Antiestrogenicity

TCDD induced 2-hydroxylation of estradiol about fourfold in female rats and twofold in male rats.[141] The induction of estradiol-2-hydroxylation was reported to be mediated by P450d (CYP1A2), although other P450 isozymes are responsible for the constitutive 2-hydroxylase activity.[112,141,142] Gierthy et al.[143] showed that TCDD induced 2-hydroxylation of estradiol eightfold and 16α-hydroxylation twofold in human MCF-7 cells. TCDD rapidly depleted MCF-7 cells of estradiol and this could be due to the highly elevated hydroxylations (20 to 40-fold) of estradiol at 2-, 4-, 6α-, and 15α-positions.[144,145] Spink et al.,[146] however, reported that cytochrome P450 1A1 catalyzes the hydroxylation of estradiol at 2-, 6α-, and 15α-positions, but not the 4-hydroxylation. The 2- and 4-hydroxylated forms of estradiol were present largely in conjugated forms.[145] Conversion of estradiol to estrone was not affected by TCDD. Gierthy and coworkers[143,144] suggested that the enhanced

metabolism, primarily to 2-OH-estradiol, accounts for the antiestrogenic activity of TCDD.

However, although the induced metabolism of 17β-estradiol may cause some of the antiestrogenic effects of TCDD at relatively high concentrations/doses, the results summarized below do not support that hypothesis[106]:

1. The antiestrogenic effects in rodents are not accompanied by decreased circulating levels of 17β-estradiol.
2. Down-regulation of nuclear estrogen receptor levels (and decreases in the 17β-estradiol-induced secretion of the 34-, 52- and 160-kDa proteins) occurs at concentrations (1p*M*) that do not cause any induction of P450 activities.
3. Down-regulation of nuclear estrogen receptor levels occurs within 2 h after treatment and only minimum induction of P450 is observed within this short time frame.
4. MCDF, which exhibits minimal CYP1A-inducing activity, also exhibits antiestrogenic activity.

However, in contrast, in adult female B6C3F1 mice, TCDD induced CYP1A1 and CYP1A2 at the lowest dose (1.5 ng/kg/d), whereas hepatic and uterine estrogen receptor levels were not altered at any of the doses tested (1.5 to 150 ng/kg/d).[147]

Both transcriptional and translational inhibitors (actinomycin D, cycloheximide) block TCDD- and MCDF-mediated down-regulation of nuclear estrogen receptor in both human MCF-7 and mouse Hepa lclc7 cells.[136,139,140] Estrogen-induced responses are thus dependent on the formation of transcriptionally active nuclear estrogen receptor complexes in target cells or tissues.[106] TCDD probably induces modulating factors that result in the down-regulation of nuclear estrogen receptor, and this may be responsible for the observed decrease in estrogen-induced responses. TCDD and progesterone exhibit comparable antiestrogenic effects *in vitro* and *in vivo*, however, probably through different mechanisms.[148,149]

Safe et al.[111] proposed the following possible mechanisms of action of TCDD and related compounds as antiestrogens:

The AhR complex may:

- Directly inhibit estrogen-induced genes
- Induce modulatory protein(s) that degrade the nuclear estrogen receptor
- Inhibit estrogen-induced gene transcription
- Inhibit the action of estrogen-induced growth factors
- Exhibit other antimitogenic activities
- Induce estrogen metabolism

Neither TCDD nor estradiol cross-react with each other's receptor.[111,121,150] The action of TCDD on the estrogen receptor is species, tissue, and site specific, which is similar to steroid hormone action.[150] The modes of action of the estrogen receptor, glucocorticoid receptor, thyroid hormone receptor, retinoic acid receptor (RAR), and AhR are analogous in that the nuclear form of the respective receptor binds to highly specific regions on a gene termed the response element. The data suggest that the binding of the AhR-nuclear protein(s)-ligand to a xenobiotic response element regulates the synthesis of estrogen receptor mRNA. This regulation is correlated with the induction of CYP1A1 and probably CYP1A2. Down-regulation of estrogen receptor is not related to changes in estradiol metabolism.[150]

White and Gasiewicz[151] showed that TCDD-activated nuclear extracts (AhR + ligand) from both mouse Hepa lclc7 cells and human MCF-7 cells specifically bound to a synthesized dioxin-responsive element (DRE)-containing region of the human estrogen receptor gene. This binding was dependent on a functional DRE. These results suggest that the TCDD-AhR complex could

act by either inhibiting the binding of other transcriptional activators to DNA, or by physically interfering with the ability of other *trans*-activators to stimulate transcription.

Other hormones can act as antiestrogens in similar ways. Retinoic acid and vitamin D_3 inhibit estrogen-induced growth of MCF-7 cells as well as estrogen-induced transcription.[152] These compounds do not compete with estradiol to bind the estrogen receptor. Retinoic acid and vitamin D_3 receptors can directly or indirectly impair the binding of estrogen receptor to the estrogen-responsive element.

d. Conclusion

Both *in vivo* and *in vitro* studies confirm that TCDD and related compounds exhibit a broad spectrum of antiestrogenic responses and that these effects are mediated through the AhR.

Although the induced metabolism of 17β-estradiol may cause some of the antiestrogenic effects of TCDD at relatively high doses, the following results do not support that hypothesis: circulating levels of 17β-estradiol are not affected by TCDD and down-regulation of the estrogen receptor occurs both earlier and at lower doses than enzyme induction. The antiestrogenic effects are not due to TCDD or estradiol cross-reacting with each other's receptor. The data suggest that the AhR-ligand complex binds to a xenobiotic response element on the estrogen receptor gene and regulates the synthesis of estrogen receptor mRNA. Also, ligands of similar receptors, retinoic acid and vitamin D_3, can impair the binding of estrogen receptor to the estrogen-responsive element.

From these studies it is clear that TCDD and other AhR agonists down-regulate estrogen receptor levels and cause antiestrogenic effects. The consequences, if any, for human health remain, however, unclear at present.

C. Experimental Evidence of Carcinogenicity

1. Introduction

Recent hypotheses that some organochlorine pesticides may play a role in the development of cancer in humans have largely been based on animal studies, but the interpretation of these laboratory findings and their relevance to human cancer is controversial. Most of the chronic toxicological and carcinogenic effects of the series of halogenated aromatic hydrocarbons, which includes PCDDs, PCDFs and PCBs, have been demonstrated in laboratory animal studies of exposure to the dioxin congener TCDD, to mixtures of 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (HxCDD), and to commercial mixtures of various PCB congeners. Little experimental data are available on the chronic effects of other members of the series, including other PCDD congeners.

Given that certain PCB congeners are now known to be more toxic than others, it is important to establish the specific isomeric composition of PCB mixtures being evaluated for carcinogenicity or for potential adverse effects in humans.[153,154] There is considerable variation in the response to these chemicals among animal species, and those organs showing the highest tissue concentrations do not necessarily show the most pathology. Animal studies have shown the liver to be the primary target organ following prolonged periods of absorption of toxic doses of these particular organochlorine compounds. The focus of this section will be on those specific organochlorine chemicals that have raised concern or been addressed in the epidemiological literature with respect to their potential relationship

to breast cancer, endometrial cancer, and endometriosis.

Many other chlorinated insecticides have been found to produce predominantly liver cancer in rodents, for example, methoxychlor, HCB, lindane, HCH, chlordane, heptachlor, heptachlorepoxide, mirex, chlordecone, aldrin, and dieldrin.[58,155–158] However, no information on estrogen-related tumors have been found for these compounds.

2. PCDDs and PCDFs

TCDD is a trace contaminant formed in the commercial synthesis of the herbicide, 2,4,5-T. TCDD, considered the most toxic of the halogenated aromatic hydrocarbons, has been used as a prototype to investigate the toxic and carcinogenic potential of PCDDs, PCDFs, and coplanar PCBs.[13,159–161] Several studies, which examined the carcinogenic effects of extended high doses of TCDD, found an increased incidence of many types of tumors, including hepatocellular carcinomas and neoplasms of the bile duct, skin, lung, palate, tongue, and thyroid.[59,162–167]

In long-term feeding studies,[103,166] the liver was the organ principally affected by TCDD exposure. TCDD produces hepatocellular carcinomas and hyperplastic (neoplastic) nodules in female, but not male, rats following continuous ingestion of a high dose level of 0.1 μg/kg/d (2200 ppt in the diet) for 2 years. This dose level also caused an increased incidence of squamous cell carcinomas of the lung, hard palate/nasal turbinate, and tongue. Lifetime ingestion of 0.01 μg/kg/d (210 ppt in the diet) or of 0.001 μg/kg/d (22 ppt in the diet) caused no significant carcinogenic response,[103] suggesting that only doses sufficient to induce severe liver toxicity increased the incidence of some types of neoplasms in the rat.

A reduced incidence of tumors of the pancreas and adrenal gland was reported by Kociba et al.[103] following TCDD administration, as well as a statistically significant dose-related decrease in tumors of estrogen-sensitive organs in female rats given the highest dose of 0.1 μg/kg/d. This included a decreased incidence of endometrial hyperplasia, subcutaneous mammary gland tumors, and pituitary adenomas, suggesting that TCDD may act through a hormonal mechanism. The reproductive organs of male rats appeared to be unaffected by the given dose levels. In addition, a single dose of 10 μg TCDD per kilogram body weight decreased dimethylbenzanthracene-induced mammary tumors in female Sprague-Dawley rats.[105] In immunosuppressed mice, a single dose of 2 to 8 μg TCDD per kilogram body weight inhibited 17β-estradiol-induced growth of implanted MCF-7 human breast cancer cells.[104]

TCDD is classified as a probable human carcinogen by the EPA[168] and as a possible human carcinogen by IARC.[155] The estimations of human risk in environmental assessments of TCDD have been based primarily on carcinogenicity studies in experimental animals, specifically the chronic bioassays of Kociba et al.[103] and the National Toxicology Program,[166] rather than on documented human health effects. Federal agencies in the U.S. extrapolate low-dose risk of TCDD exposure in humans on the basis of animal tumor data obtained at high doses (usually 100,000 times higher): an underlying assumption of this method, which uses a nonthreshold linearized multistage model for estimating human cancer risk,[169] is that TCDD behaves as a tumor initiator, despite the fact that most mutagenicity and carcinogenicity data suggest that TCDD is nongenotoxic and induces tumors in animals through an epigenetic mechanism.

A biologically based model for the risk assessment of receptor-mediated, nonmutagenic carcinogens may be more appropriate,[169] especially given that concentrations of the TCDD isomer in adipose tissue from the general population have been non-

detectable or in the low nanogram per kilogram adipose tissue range.[170] In evaluating potential risk to humans, it may also be relevant that rodents have an unusually high rate of spontaneous liver tumors in comparison with humans.[171]

Gold et al.[172] used the human exposure/rodent potency (HERP) index of possible hazard rather than a direct estimate of risk to assess human risk at low dose for chemicals in the human diet. They concluded that, relative to the large background of naturally occurring carcinogens in typical portions of common foods, the residues of synthetic pesticides or environmental pollutants do not rank high.

3. PCBs

Evaluations of PCBs for carcinogenicity must take into account the fact that the composition of commercial PCB mixtures is different from that of the mixtures present in the environment due to metabolism of PCBs during passage through the food chain. Most early animal studies used commercial mixtures of PCBs, which were found to affect reproduction and the immune response and to induce neoplastic nodules in the liver and in some cases hepatocellular carcinomas.[170] However, when different mixtures of PCBs were studied the results were inconsistent, and more recently some of the isomers of the PCB mixture were found to be more toxic than others.[153] The ability of PCB mixtures to generate liver tumors appears to be related to the degree of chlorination of the mixture,[173] with the most potent being Aroclor 1260, Clophen A 60, and Kanechlor 500. Whereas TCDD causes a variety of tumors in experimental animals, only liver tumors[24,174-179] and, in one study,[180,181] tumors of the stomach have been induced with extended administration of PCBs. Neoplasms of the liver appear relatively unaggressive and rarely if ever metastasize to distant organs.[24]

The PCB mixture Kanechlor 400 produced multiple adenomatous nodules, which appeared to be benign, in all female rats ingesting more than 1200 mg over a period of at least 400 d. None of the treated male rats showed such neoplastic changes.[175]

Evidence of the potential for carcinogenicity of the Aroclor mixtures was also presented in the 1970s. Kimbrough et al.[177] exposed female Sherman rats to dietary levels of 100 ppm of a commercial PCB mixture of Aroclor 1260 for 21 months. They found 26 hepatocellular carcinomas in 184 exposed rats (14.1%) compared with one hepatocellular carcinoma among 173 controls (0.6%), as well as an increase in neoplastic nodules among exposed rats. In another study,[176] hepatomas ranging from 0.1 to 1.5 cm in diameter were present in 40% (9 of 22) of mice fed 300 ppm Aroclor 1254 in the diet for 11 months, and adenofibrosis was observed in all 22 livers. No control mice had hepatomas or adenofibrosis. Although lesions were reported in a few other organs, their incidence was not significantly higher than that in controls, indicating that any potential carcinogenic effect of PCBs is confined primarily to the liver.

When Fischer 344 rats were given different dosage levels of 25, 50, and 100 ppm Aroclor 1254 for 104 to 105 weeks, proliferative lesions of the liver, including carcinoma and hyperplastic nodules, occurred in 7 of 24 (29%) PCB-treated animals but were absent in controls.[182] Although initially considered not statistically significant, a reanalysis[183] indicated that this difference was indeed significant ($p < 0.05$) with a dose-related trend ($p < 0.01$). Moreover, a reexamination of the stomachs of rats revealed six adenocarcinomas of the stomach among 144 Fisher F344 rats exposed to Aroclor 1254 (4.2%), as well as a high incidence of squamous metaplasia in the epithelium of the stomach.[180] The investigators concluded that